GROWTH RATE AND TOXICITY OF *PRYMNESIUM PARVUM* AND *PRYMNESIUM PATELLIFERUM* (HAPTOPHYTA) IN RESPONSE TO CHANGES IN SALINITY, LIGHT AND TEMPERATURE

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Toxic blooms consisting of *Prymnesium parvum* CARTER and *Prymnesium patelliferum* GREEN, HIBBERD & PIENAAR have caused variable degrees of mortality of farmed fish in the Sandsfjord system, southwestern Norway annually between 1989 and 1996. Cultures of *P. parvum* and *P. patelliferum* were established from water samples during the bloom in the Sandsfjord system in 1993. In order to investigate possible differences in reproduction rates and autecology between the two species, growth as a function of different salinity, photon fluence rates (PFR) and temperatures of the two Norwegian strains was compared with the corresponding growth of strains of *P. parvum* from England and Denmark, and strains of *P. patelliferum* from Australia and England. The toxicities of the same strains grown under varying salinity, light and temperature conditions, were also compared. Maximum growth rates were generally higher in *P. patelliferum* than in *P. parvum*, but strain differences within the species varied significantly. Both optimal growth conditions and toxicity differed greatly between the strains, and seem to be more linked to the individual strains than to species. Furthermore, there was no apparent relationship between toxicity and salinity, temperature or PFR. Thus, there were no marked differences in autecological and toxic properties between *P. parvum* and *P. patelliferum* apparent from this investigation.

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INTRODUCTION

At present there are ten recorded species of *Prymnesium* (JORDAN & GREEN 1994; PIENAAR & BIRKHEAD 1994). Some are of doubtful taxonomic status, but of those that are well documented, *Prymnesium calathiferum* CHANG & RYAN, *Prymnesium parvum* and *Prymnesium patelliferum* are known to be toxic (see review by EDVARDSEN & PAASCHE 1998). Every summer during the period of 1989 to 1996, mixed blooms of the two species *P. parvum* and *P. patelliferum* have occurred in the Sandsfjord system, southwestern (SW) Norway (EIKREM & THRONDSEN 1993; EDVARDSEN & PAASCHE 1998). The blooms have resulted in a variable degree of mortality of farmed fish (EIKREM & THRONDSEN 1993; LIE & MAGNESEN 1993), and the two species thus represent a constant problem for fish-farmers in the area.

The first recorded event of fishkills caused by *P. parvum* took place in Workum See in Holland in 1920 (LIEBERT & DEERNS 1920). Since then there have been several reports of fish mortality caused by this species from various parts of the world (see reviews by MOESTRUP 1994 and EDVARDSEN & PAASCHE 1998). GREEN & al. 1982 (with reference to VALKANOV 1964) indicated that *P. patelliferum* should also be regarded as toxic. ARLSTAD (1991) documented the toxic potential of this species by testing it against the dinoflagellate *Heterocapsa triquetra* STEIN. Initially the fishkills during the *Prymnesium* blooms in the Sandsfjord system were ascribed to *P. parvum* only. It was later shown, however, that the blooms contained both *P. parvum* and *P. patelliferum* (EIKREM & THRONDSEN 1993). The toxic potency of a *P. patelliferum* strain from the area was demonstrated as well (LARSEN & al. 1993).

Prymnesium parvum and *P. patelliferum* are distinguished by minor differences in the morphology of the organic scales covering their cell surface (GREEN & al. 1982). Recognition of the differences requires the use of transmission electron microscope (TEM). It is possible, therefore, that some blooms of *P. parvum*, identified only by light microscopy, may have been blooms of *P. patelliferum*.

Since *P. parvum* was first recognized as ichthyotoxic, this species and its toxins have been amply studied.

The exotoxins produced by *P. parvum* are composed of several components that may induce a variety of physiological responses, such as ichthyotoxic, cytotoxic, neurotoxic and antibacterial activity (SHILO 1971; MELDAHL & FONNUM 1993; IGARASHI & al. 1996). Nutrient conditions, growth phase and the presence or absence of potent cofactors are among factors influencing the toxicity extensively (e.g. YARIV & HESTRIN 1961; SHILO 1971; LARSEN & al. 1993). Investigations of how changing salinity and light conditions influence the toxicity of *P. parvum* have given contradicting results without clear conclusions as to whether high or low salinities and high or low irradiances enhance toxicity (SHILO & ASCHNER 1953; REICH & PARNAS 1962; ULITZUR & SHILO 1964; RAHAT & JAHN 1965; PADAN & al. 1967; SHILO 1967; PADILLA 1970; DAFNI & al. 1972; ARLSTAD 1991; KVERNSTUEN 1993). Correlations between temperature and toxin production have not been found (SHILO 1971).

The effects of environmental conditions on growth of *P. parvum* have also been subject to much research (MCLAUGHLIN 1958; RAHAT & JAHN 1965; PADILLA & al. 1967; PADILLA 1970; SHILO 1971; DAFNI & al. 1972; HOLDWAY & al. 1978; BRAND 1984; DICKSON & KIRST 1987; GUO & al. 1988; ARLSTAD 1991; LARSEN & al. 1993). Most of the autecological studies have involved only investigations of the influence of salinity on growth rates, and have shown that *P. parvum* is extremely euryhaline. Disagreements on the optimum salinity for growth do exist however. Information about how light and temperature influence reproduction rates, is more sparse, but indicates that *P. parvum* prefers high irradiances, and is able to grow over a wide range of temperatures.

Only a limited amount of information on how growth and toxicity in *P. patelliferum* is influenced by environmental conditions has been published. It has been claimed that it grows faster and is less toxic than *P. parvum* (ARLSTAD 1991; MELDAHL & al. 1994). LARSEN & al. (1993) showed, however, that the two species can be equally toxic to *Artemia* nauplii and that growth rate within *P. patelliferum* can vary substantially.

Common to most of the previous work on growth and toxicity in *Prymnesium*, are that the conclusions are based on results from one strain only. In cases where more than one strain have been used, the differences are claimed to be negligible, not quantified or not mentioned at all.

The aim of this study was to investigate several strains of both *P. parvum* and *P. patelliferum* in order to reveal possible differences in autecological and toxic properties between the two species. Do the two species have different tolerance limits and/or different requirements for optimum growth? Can differences in maximum growth rates and toxicity between the two species be documented? How is the toxicity of the two species influenced by variable salinity, irradiance and temperature conditions? Three different experiments examining growth rates and toxicity at varying salinities, irradiances and temperatures in three strains of *P. parvum* and three strains of *P. patelliferum*, were carried out. Growth rates were determined by daily *in-vivo* fluorescence measurements. A bioassay using nauplii of the crustacean *Artemia* as a test organism was executed to assess the toxicity of the algal cultures.

MATERIAL AND METHODS

Algal strains and standard culture conditions

The algal strains used in this study are listed in Table 1. Species designations were confirmed by means of TEM for all strains. All strains except RL10parv93, was made axenic by antibiotic treatment (DROOP 1967). Sterility tests (i.e. transfer of one drop of the culture into a sterility test medium, DROOP 1967) were performed at the axenic cultures at each transfer (every three to four weeks) in order to ascertain they stayed axenic. It has been shown earlier that growth rates in *P. parvum* and *P. patelliferum* obtained in the presence of bacteria do not differ significantly from those of bacteria free cultures (LARSEN & al. 1993). However, whether the presence of bacteria influences the toxicity of *P. parvum* and *P. patelliferum* we do not know. Stock cultures were grown in IMR medium (EPPLEY & al. 1967) prepared from filtered (0.45 µm cellulose nitrate filters, Sartorius), autoclaved seawater diluted with ion-free water (Milli-Q) to 8 practical salinity units (psu). Nutrients, vitamins and chelated trace metals were added at half the original recommended concentrations (1/2 IMR). Stock cultures were

Table 1. Algal strains used in this study.

Species	Origin	Culture collection	Culture code	Isolated by, year
Prymnesium patelliferum	The Sandsfjord system, Norway	Bergen	RHpat93[1)]	A. Larsen, 1993
Prymnesium patelliferum	The Fleet, Dorset, England	Plymouth	PLY527[1)]	D. Hibberd, 1976
Prymnesium patelliferum	Norman Bay, Australia	Scandinavian, Copenhagen	K0252[1)]	Ö. Moestrup, 1987
Prymnesium parvum	Flade Sø, Thy, Denmark	Scandinavian, Copenhagen	K0081[2)]	T. Christensen, 1985
Prymnesium parvum	R. Blackwater, England	Plymouth	PLY94[1)]	D.R.W. Butcher, 1952
Prymnesium parvum	The Sandsfjord system Norway	Bergen	RL10parv93[1)]	A. Larsen, 1993

[1)]: The culture is clonal [2)]: The culture is not clonal

grown at 15 °C under white fluorescent light with a photon fluence rate (PFR) of 150 µmol m^{-2} s^{-1} and a 14:10 h L:D cycle. These are growth conditions that previously have shown to give good (although not optimal) growth in some strains of the species (Larsen & al. 1993; personal observations).

Growth and toxicity

Three experiments were executed to study the effects of different salinity, light and temperature conditions on growth and toxicity of the six *Prymnesium* strains. In all three experiments batch cultures were grown in borosilicate tubes in 35 ml 1/2 IMR medium. Before adding nutrients the seawater was diluted to desired salinity with ion-free water (milli-Q), measured by Hand Refractometer. Triplicate growth tubes were placed in racks on shelves in climate rooms with temperatures and light conditions as indicated below. Photon fluence rates were measured within tubes with a quantum meter (QSL-100, Biospherical Instruments, San Diego, Ca, USA). The tubes within each light regime were rotated manually once a day to obtain equal light conditions. Inoculum cultures were grown under conditions similar to the test conditions.

In the salinity experiment growth-medium was diluted with ion-free water (milli-Q) to the following salinities: 3, 8, 18, 30 psu. Average PFR was 230 µmol m^{-2} s^{-1}, the L:D cycle was 12:12 h, and the temperature 25 °C (±1.5 °C)

In the light experiment the growth tubes were placed in racks on shelves in a culture room at following PFRs (average values) 25, 65, 100, 150 and 250 µmol m^{-2} s^{-1} on a 12:12 h L:D cycle. The temperature was 25 °C (±1.5 °C) and the salinity 12 psu.

In the temperature experiment the growth tubes were placed in racks on shelves in 4 different culture rooms at the following temperatures: 5, 11, 15 and 30 °C. The average PFRs was 230 µmol m^{-2} s^{-1}, the L:D cycle 14:10 h, and the salinity 12 psu.

The chosen standard level of irradiance (230 µmol m^{-2} s^{-1}) was selected because one of our strains which was also used in a previous study, required approximately 200 µmol m^{-2} s^{-1} for light saturated growth, and none of the strains was inhibited by this irradiance level (Larsen & al. 1993). Similarly some strains (in the same study) indicated that 25 °C is close to optimal temperature for growth. Blooms of *P. parvum* and *P. patelliferum* have been reported from localities in which temperatures often get close 25 °C when irradiances are high (i.e. shallow ponds or narrow brackish water layer, e.g. Reich & Aschner 1947; Kaartvedt & al. 1991). The chosen levels might thus be close to natural ones.

Artemia *bioassay*

Toxicity tests were carried out with a strain of the crustacea *Artemia* sp. (Creasel, Deinze, Belgium). In late exponential growth phase, the cell number of the algal cultures was determined using an electronic particle counter (Coulter Counter, model ZM, Coulter Electronics Ltd.) and toxicity tests performed. The protocol developed at The Artemia Reference Centre in Belgium (Vanhaecke & al. 1981) was followed. Cultures of *Isochrysis galbana* Parke and 70 % seawater were used as controls. The mortality in the controls was less than 1 %. To make sure that the toxicity test-results were reproducible, the sensitivity of the *Artemia* nauplii was checked with sodium lauryl-sulphate (SDS) during the whole experiment period (Vanhaecke & al. 1981). During the salinity and light experiments the *Artemia* nauplii were incubated at 25 °C while it was incubated at 15 °C in the temperature experiment. *Artemia* spp. is common as test organisms in ecotoxicological bioassays (Persoone & Wells 1987) and was chosen for the toxicity experiments as it has shown to be sensitive to toxins produced by hatophytes (Edvardsen & Paasche 1992; Larsen & al. 1993; Meldahl & al. 1994; Rhodes & al. 1994; Edvardsen & al. 1996).

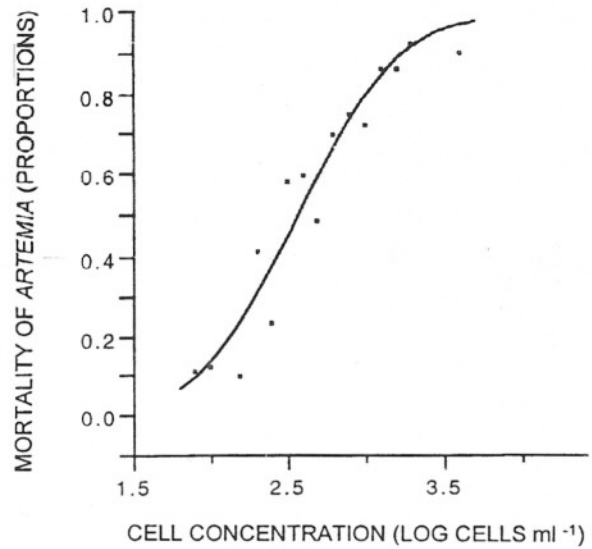

Fig. 1. The proportion of dead *Artemia* nauplii (mortality) as a function of log cell concentration of *Prymnesium*. The example is from the light experiment (strain PLY94 grown at 250 µmol m^{-2} s^{-1}). See text for further explanation.

Calculations and statistical analyses

Cell abundance was estimated by inserting the growth tubes into a fluorometer (10-AU Fluorometer, Turner Designs, Sunnyvale, Ca, USA) as described by Brand & Guillard (1981). Exponential growth rates in the unit divisions per day (div. day^{-1}) were calculated as

$$k = \log (N_1 / N_0) (3.322/t_1 - t_0)$$

where N_0 and N_1 is cell density (measured as fluorescent units) at time 0 and 1 respectively, and t is time in days.

The algal cell density giving 50 % mortality (LC50) of the *Artemia* nauplii during 24 h was calculated by assuming that the sensitivity of individual *Artemia* nauplii to *Prymnesium* toxins is log-normally distributed with mean log LC50 and standard deviation s. The expected proportion of dead *Artemia* nauplii at algal concentration x, is then:

$$p(x) = Z((\log x - \log LC50) / s) \qquad (1)$$

where Z is the cumulative standardised normal distribution function. The parameters log LC50, s and 95 % confidence intervals were estimated by fitting eq. 1 to observed proportions of dead *Artemia* (termed mortality) by non-linear regression (Zar 1984 p. 349-351, for an example see Fig. 1).

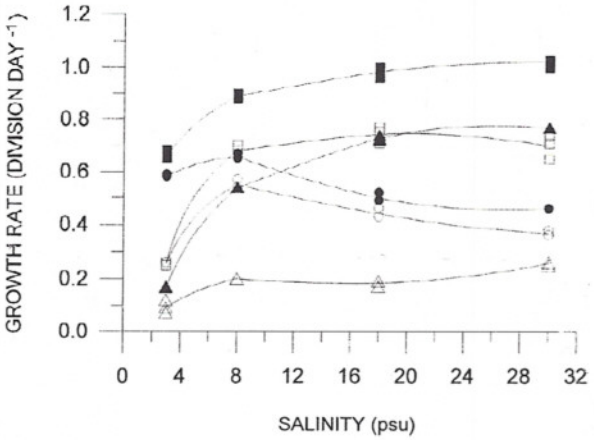

Fig. 2. Exponential growth rate as a function of salinity in three strains of *P. parvum* (RL10parv93 (○), K0081 (□), PLY94 (△) and three strains of *P. patelliferum* (RHpat93 (●), K0252 (■), PLY527 (▲)). n = 3 for all strains at each psu.

LC50-values and confidence intervals were estimated on the basis of all parallels together. The LC 50 value is an inverse measure of the toxic agent's toxicity to the test organism.

The data were analysed by Analysis of Variance (ANOVA, Zar 1984, p. 206-226) and Tukey-Kramer's HSD-test for multiple comparison among means (Zar 1984, p. 203-205). LC50 24 h values were log 10 transformed prior to the statistical analysis.

RESULTS

Salinity experiment

Growth rates as function of salinity are shown in Fig. 2. In *P. parvum* and *P. patelliferum* isolated from the Sandsfjord system, Norway (RL10parv93 and RHpat93), highest growth rates were achieved at 8 psu, in *P. parvum* from Denmark (K0081) at 18 psu and in *P. parvum* from England (PLY94), and in *P. patelliferum* from England and Australia (PLY527 and K0252) at 30 psu. Lowest growth rates were obtained at 3 psu in all strains except for Norwegian *P. patelliferum*, in which the growth was more reduced

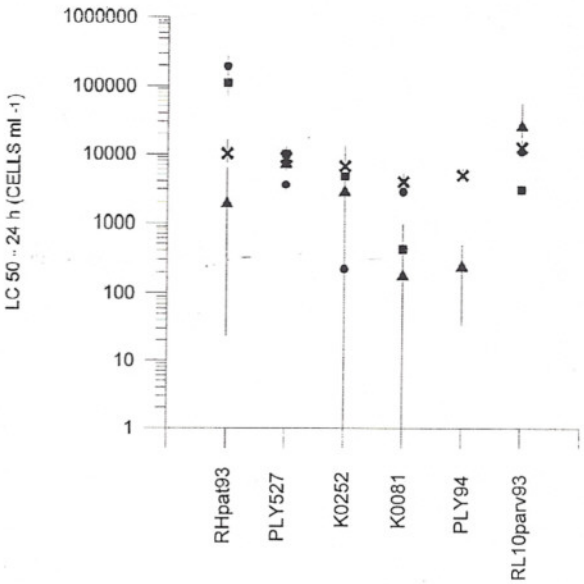

Fig.3. LC50-24h-values for *Artemia* nauplii when exposed to the six *Prymnesium* strains grown at 3 (■), 8 (●), 18 (▲) and 30 (×) PSU. Vertical bars indicate 95 % confidence intervals, based on 3 parallels with 10 nauplii in each parallel.

at both 18 and 30 psu. Comparison of maximum growth rates (MGR), which are given for each strain in Table 2, show that *P. patelliferum* grew faster than *P. parvum* ($p = 0.01$, ANOVA). Maximum growth rates varied significantly between the strains, however ($p < 0.000001$). Differences in MGR were found between all strains at $p < 0.05$ (Tukey-Kramer HSD), except between *P. parvum* and *P. patelliferum* isolated from the Sandsfjord system, Norway (RL10parv93 and RHpat93), and between *P. parvum* from Denmark (K0081) and *P. patelliferum* from England (PLY527).

The toxicity to *Artemia* nauplii varied considerably in some of the strains when grown at different salinities (Fig. 3). The variations were not systematic between the different strains, however, and no significant effect of salinity on toxicity was demonstrated ($p = 0.81$, ANOVA). Maximum average LC50 values for each

Table 2. Maximum average growth rates (div. day^{-1}) in each clone, in each of the three experiments. Parentheses indicate lowest and highest value of three parallels.

Strain	Salinity experiment	Light experiment	Temperature experiment
Prymnesium patelliferum:			
RHpat93	0.67 (0.65-0.67)	0.55 (0.55-0.55)	0.52 (0.51-0.52)
PLY527	0.77 (0.77-0.77)	0.63 (0.62-0.64)	0.53 (0.51-0.55)
K0252	1.02 (1.00-1.04)	0.97 (0.94-1.00)	1.15 (1.14-1.17)
Prymnesium parvum:			
K0081	0.74 (0.71-0.77)	0.54 (0.51-0.58)	0.38 (0.38-0.39)
PLY94	0.26 (0.25-0.26)	0.13 (0.12-0.14)	0.19 (0.17-0.22)
RL10parv93	0.55 (0.54-0.57)	0.38 (0.36-0.39)	0.38 (0.36-0.39)

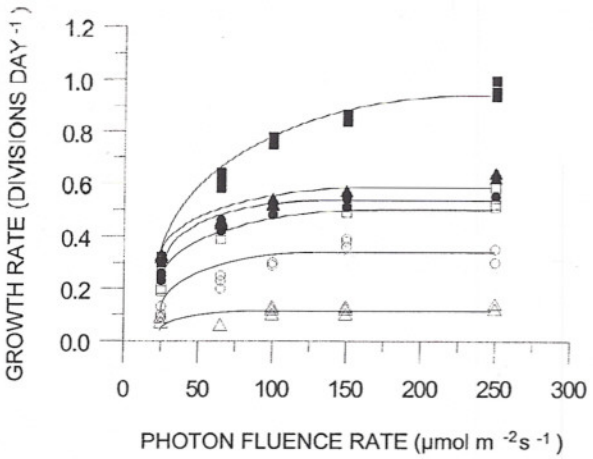

Fig. 4. Exponential growth rate as a function of photon fluence rate (PFR) in three strains of *P. parvum* (RL10parv93 (○), K0081 (□), PLY94 (△) and three strains of *P. patelliferum* (RHpat93 (●), K0252 (■), PLY527 (▲)). n = 3 for all strains at each PFR.

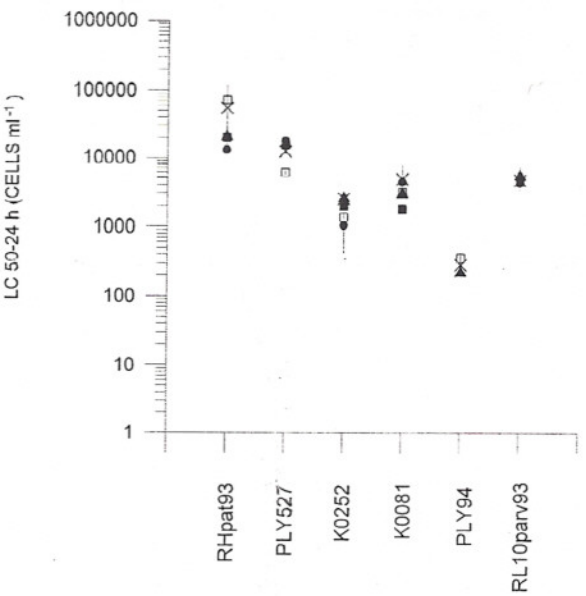

Fig.5. LC50-24h-values for *Artemia* nauplii when exposed to the six *Prymnesium* strains grown at 25 (■), 65 (●), 100 (▲), 150 (×) and 250 (□) PFR. Vertical bars indicate 95 % confidence intervals, based on 3 parallels with 10 nauplii in each parallel.

strain are given in Table 3 and show that one strain of each species (K0081 and K0252) exhibited highest maximum toxicity and one strain of each species the lowest (PLY527 and RL10parv93). However, a comparison of toxicity between all clones (based on LC50 24 h for all salinities, Table 3), showed significant difference in toxicity only between Danish *P. parvum* (K0081) and Norwegian *P. patelliferum* (RHpat93, $p < 0.05$, Tukey-Kramer HSD), and in general no difference in toxicity between the two species was documented ($p = 0.14$, ANOVA). One *P. parvum* strain (PLY94) never grew dense enough at 3 and 8 psu to perform the toxicity test.

Light experiment

The photon fluence rate (PFR) required for light saturated growth differed between the strains (Fig. 4). The saturation PFR was estimated to be approximately 200 µmol m^{-2} s^{-1} for *P. patelliferum* from Australia (K0252), around 100 µmol m^{-2} s^{-1} for the four following strains: *P. parvum* from Sandsfjord (RL10parv93) and Denmark (K0081), *P. patelliferum* from England (PLY527) and Sandsfjord (RHpat93), and around 65 µmol m^{-2} s^{-1} for *P. parvum* isolated from England (PLY94). Maximum growth rates, which proved to be significantly different between the species, with *P. patelliferum* growing faster than *P. parvum* ($p = 0.003$, ANOVA), are shown in Table 2. A comparison of MGR between all strains showed non-significant difference in MGR between the Norwegian and English *P. patelliferum* (RHpat93 and PLY527) and between Danish *P. parvum* (K0081) and *P. patelliferum* from England (PLY527). The rest of the strains grew significantly differently from each other ($p < 0.05$, Tukey-Kramer HSD).

Table 3. Maximum average toxicity (LC50-24h) in each clone (lowest values represent highest toxicity), in each of the three experiments. Parentheses show 95 % confidence intervals.

Strain	Salinity experiment	Light experiment	Temperature experiment
Prymnesium patelliferum:			
RHpat93	1995 (23-6457)	13183 (14125-34674)	6310 (5370-7586)
PLY527	3548 (2951-3981)	6026 (5129-6918)	126 (107-145)
K0252	219 (1-977)	1047 (417-1862)	537 (308-661)
Prymnesium parvum:			
K0081	186 (1-1000)	1820 (1514-2188)	427 (331-550)
PLY94	251 (33-501)	234 (209-263)	661 (575-741)
RL10parv93	3090 (2570-3631)	4266 (3311-5495)	776 (537-1096)

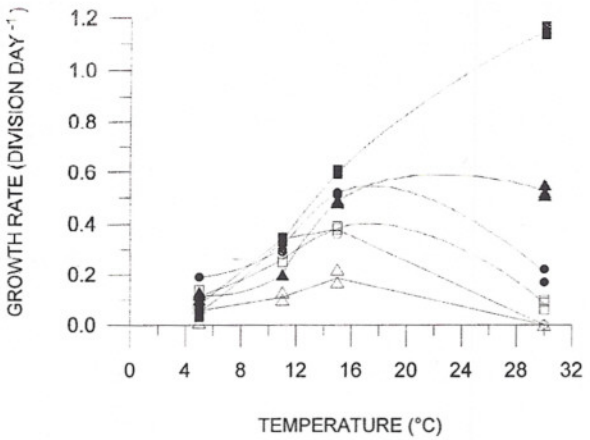

Fig. 6. Exponential growth rate as a function of temperature in three strains of *P. parvum* (RL10parv93 (○), K0081 (□), PLY94 (△) and three strains of *P. patelliferum* (RHpat93 (●), K0252 (■), PLY527 (▲)). n = 3 for all strains at each temperature.

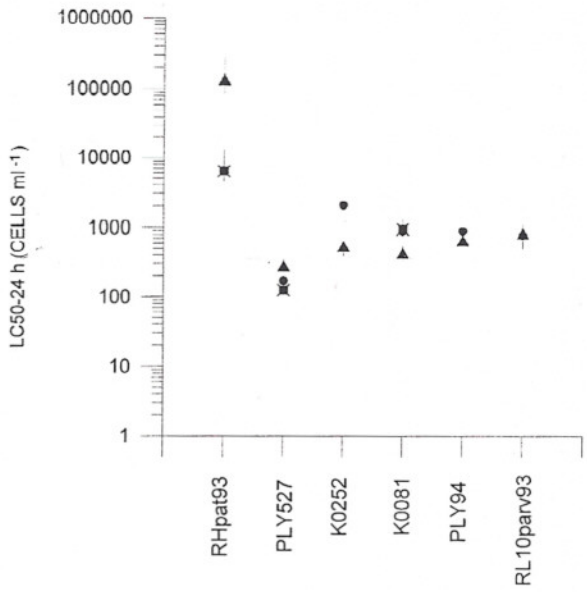

Fig.7. LC50-24h-values for *Artemia* nauplii when exposed to the six *Prymnesium* strains grown at 5 (■), 11 (●), 15 (▲) and 30(×) °C. Vertical bars indicate 95% confidence intervals, based on 3 parallels with 10 nauplii in each parallel.

The photon fluence rate did not influence the overall toxicity within *P. parvum* and *P. patelliferum* ($p = 0.80$, ANOVA, Fig. 5). However, *P. parvum* appeared to be more toxic than *P. patelliferum* in this experiment (ANOVA, $p = 0.01$, Table 3). A significant difference in toxicity between the strains was also shown (ANOVA, $p < 0.00001$). Ranking the *Prymnesium* strains according to toxicity (from highest to lowest) gave the following order: PLY94, K0252, K0081, RL10parv93, PLY527, RHpat93. When comparing all LC50-24 h with one another, all pairs, except the three following ones: RHpat93-PLY527, K0252-K0081 and K0081-RL10parv93, differed significantly (Tukey-Kramer at p-level 0.05). Strain PLY94 exhibited highest and strain RHpat 93 lowest maximum toxicity (Table 3). Strain PLY94 never grew densely enough at 25 and 65 PFR to perform the toxicity test.

Temperature experiment

Maximum growth rate was obtained at 15 °C gave for all strains, except for English and Australian *P. patelliferum* (PLY527 and K0252) which obtained their highest growth rates at 30 °C (Fig. 6). *Prymnesium patelliferum* showed significantly higher growth rates than *P. parvum* ($p = 0.0003$, ANOVA, Table 2). The comparison of MGR between all strains showed that except for Norwegian and English *P. patelliferum* (RHpat93 and PLY 527), and Danish and Norwegian *P. parvum* (K0081 and RL10parv93), all strains exhibited different maximum growth rates ($p < 0.05$, Tukey-Kramer HSD).

Toxicity to *Artemia* nauplii was significantly lower for the *P. patelliferum* isolated from the Sandsfjord system (RHpat93) than all the other strains which in turn did not differ significantly from one another (Tukey-Kramer at 0.05 p-level, Fig. 7). Neither difference in toxicity between *P. parvum* and *P. patelliferum* (ANOVA, $p = 0.91$), nor any effect of temperature on toxicity to *Artemia* were demonstrated (ANOVA, $p = 0.70$). Strain PLY94 and strain RL10parv93 grown at 5 °C and 30 °C, strain K0081 grown at 30 °C and strain K0252 grown at 5 °C never grew dense enough to be used in toxicity tests.

DISCUSSION

Growth

In agreement with previously published documentation of wide osmotic tolerances in *P. parvum* (e.g. McLaughlin 1958; Padilla 1970; Brand 1984; Dickson & Kirst 1987) and *P. patelliferum* (Arlstad 1991; Larsen & al. 1993), all *Prymnesium* strains used in our experiments, grew well over a wide range of salinities. No documentation of difference in optimal salinity between the two species emerged from our results, but a great variety in optimal salinity for growth between the strains within the species, was demonstrated. The Norwegian *P. parvum* and *P. patelliferum* strains, both obtained maximum growth rates at 8 psu, the Danish *P. parvum* strain at 18 psu, and *P. parvum* from England and *P. patelliferum* from England and Australia, at 30 psu. In contrast to the general agreement of a great tolerance to salinity variations, the reports of optimum

salinity for growth are more ambiguous. Salinities from 6 to 41 psu have all previously been claimed to give maximum growth rates in *P. parvum* (PADILLA & al. 1967; PADILLA 1970; HOLDWAY & al. 1978; BRAND 1984; DICKSON & KIRST 1987; KAARTVEDT & al. 1990; ARLSTAD 1991). The more sparse investigations on *P. patelliferum* with respect to optimum salinity conditions also show disagreement (ARLSTAD 1991; LARSEN & al. 1993). Many of the previous studies are characterized by having included only one strain (or possibly two) in the experiments. As our results demonstrated great variations in optimum salinities between strains within both species, there is reason to believe that earlier discrepancies in reported salinity optima for *P. parvum* and *P. patelliferum* may be due to use of different strains of the two species, and presumably different ecotypes, in the various studies. In our experiments all strains survived all salinities from 3 to 30 psu, and no clear differences in salinity tolerance between *P. parvum* and *P. patelliferum* were thus documented.

Prymnesium parvum and *P. patelliferum* both thrive over a wide temperature range. Blooms of *Prymnesium parvum* have been recorded from 5 °C (KRASCHNOCHEK & ABRAMOVITCH 1971) to 30 °C (SHILO 1972), and broad temperature tolerances in both *P. parvum* and *P. patelliferum* have been documented experimentally (LARSEN & al. 1993). All strains used in the present study, except for one (PLY94), tolerated temperatures from 5 °C to 30 °C. The eurytherm property of both species was thus supported. In the closely related haptophyte genera *Phaeocystis* LAGERHEIM, different temperature tolerance and temperature optima supported separation of the three species, *P. globosa* SCHERFFEL, *P. pouchetii* (HARIOT) LAGERHEIM and *P. antarctica* KARSTEN (BAUMANN & al. 1994). A similar support for separation of *P. parvum* and *P. patelliferum* at the species level was not obtained from the results of our experiments. All strains except for the Australian *P. patelliferum* (K0252) exhibited maximum growth at 15 °C, and as mentioned above only one *P. parvum* strain (PLY 94) did not survive at the whole temperature range used in our study. Optimum light conditions for growth differed between the Australian *P. patelliferum* and the other strains, as it required twice, or more, the PFR needed by these to obtain saturated growth. LARSEN & al. (1993) suggested a genetic differentiation between the Australian and northern European strains in response to differences in climate conditions. This is supported by our present study. In summary, however, neither irradiances nor temperature tolerances and optima documented autecological differences between *P. parvum* and *P. patelliferum*.

In phytoplankton, which often reproduce asexually, acclimated growth rates, which can vary substantially within species, have commonly been used to detect genetic variability (e. g. BRAND 1981; BRAND & al. 1981; BRAND 1982; GALLAGHER 1982; BLEIJSWIJK & al. 1994; PAASCHE & al. 1996). Inclusion of several clonal cultures of a species is thus necessary to obtain a correct picture of its reproductive ability (BRAND 1989). Our three experiments carried out with three strains of each species, similarly indicate significant genetic variation within both of the *Prymnesium* species studied. Maximum growth rate differences, as seen in our three experiments varied from 0-315 % and 32-121 % in *P. parvum* and *P. patelliferum* respectively. In spite of the great intraspecific growth rate differences, all three experiments showed that *P. patelliferum*, as a species, grew faster than *P. parvum*. When comparing maximum growth rates of the two species isolated from the same geographical area (the strains from the Sandsfjord system, Norway), significant differences were found in two of three experiments.

Toxicity

Previous reports claiming *P. parvum* to be more toxic than *P. patelliferum* (ARLSTAD 1991; MELDAHL & al. 1994) are not supported by our investigations as significantly difference in toxicity between the two species was documented only in one of three experiments. However, we observed different levels of toxicity between strains within each species, as earlier demonstrated for cyanophytes, dinoflagellates and diatoms (e.g. SKULBERG & al. 1993; BOMBER & al. 1989; LUNDHOLM & al. 1994). Comparisons of which strain that exhibit highest toxicity shows that a ranking from highest to lowest maximum toxicity is different in each experiment. This is probably due to different responses to variation in environmental conditions between the strains.

Variable salinity, light or temperature conditions did not prove to have significant effects on the toxicity in either of the two *Prymnesium* species used in the current experiments. Earlier studies have shown decreasing toxicity with increasing salinity in *P. parvum* (PADILLA & al. 1967; SHILO 1967). Other investigations, however, have demonstrated that up to a certain salinity there is a decrease in toxicity as salinity increases, then, as the salinity is further raised, an increase in toxic effects can be found (ULITZUR & SHILO 1964; PADILLA 1970). In the closely related *Chrysochromulina polylepis* MANTON & PARKE, which has a toxin complex very similar to that of *Prymnesium* (for review, see EDVARDSEN & PAASCHE 1998), a weak positive correlation between toxicity and salinity has been shown (EDVARDSEN & al. 1996). Documented effects of variations in light conditions on the toxicity of *P. parvum* are also contradictory. Most authors claim that toxin production is dependent on light (SHILO & ASCHNER

1953; PADAN & al. 1967; SHILO 1967; DAFNI & al. 1972), but some have found it to be higher in the dark (RAHAT & JAHN 1965). Temperature may have an impact on toxin production in species of other algae classes such as dinoflagellates (e.g. OGATA & al. 1987) and diatoms (e.g. LEWIS & al. 1993; LUNDHOLM & al. 1994), but our results are in agreement with ULITZUR & SHILO (1964) who found no difference in toxin production in any strains when grown at high or low temperatures. Similarly, temperature variation does not seem to affect toxin production in *C. polylepis* (EDVARDSEN & al. 1996).

The differences between the results of the various studies may be due to several circumstances, e. g. employment of different test methods or test organisms, measuring different components of the toxin complex or the presence of different ions in growth media (SHILO 1971). Toxins of *P. parvum* and *P. patelliferum* have been shown to be inactivated by light and to be sensitive to temperature (REICH & PARNAS 1962; RAHAT & JAHN 1965; PASTER 1968; KVERNSTUEN 1993), and differences in experimental conditions might thus have caused variable results. In addition, the current experiments have showed that different strains show different responses to changes in salinity, irradiance and temperature. In the light experiment for example, the highest toxicity in the English *P. patelliferum* strain was obtained at highest PFR, while it gave lowest toxicity in the Norwegian *P. patelliferum* strain. The use of different strains, or ecotypes, may account for some of the disagreements.

Summary

In summary, our investigation has shown differences in requirements for optimum growth, and tolerances to variations in salinity, irradiance and temperature between individual clones of *P. parvum* and *P. patelliferum*. This probably reflects genetic differences, possibly arising at least in part from geographical origin. Within the framework of such clonal variation, however, we were unable to find any significant autecological differences between the two species. Although strains of both species isolated from the same geographical area were included, our investigations did thus not answer the question, raised by LARSEN & al. (1993), of why two so similar species occupy the same ecological niche. This might be explained, however, by recent evidence that suggest that *P. parvum* and *P. patelliferum*, which are known to be close genetically (LARSEN & MEDLIN 1997), are both part of the same haplo-diploid life cycle (Larsen & Edvardsen, unpublished). Indeed, VALERO & al. (1992) have remarked that a haplo-diploid life cycle is difficult to explain if there is no difference between the ecological niches of the two generations. However, we know little about the ecological requirements, apart from preferred salinity, temperature and irradiances, of *P. parvum and P. patelliferum*. There may thus be differences, like dissimilar nutrient requirements, between them that have yet to be detected.

We found no differences in toxicity between *P. parvum* and *P. patelliferum* though we did find differences in toxicity between clones. In any one clone there was no predictable effect on toxicity by varying salinity, light or temperature. Growth phase and nutrient status thus probably have a greater impact on toxicity (e.g. SHILO 1971; LARSEN & al. 1993; MELDAHL & al. 1994) and possibly mask any variation due to factors that we have studied.

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